FINAL REPORT

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OBJECTIVE: To investigate the effects of moderate hydrostatic pressures on signal transduction we have used the A_1 adenosine receptor - inhibitory G protein (G_i) - adenylyl cyclase system in two species of scorpaenid fish which have served as a model for the study of pressure adaptation. These species, Sebastolobus alascanus and S. altivelis, have similar body temperatures, but dwell at different depths, and thus experience different hydrostatic pressures. The experiments were designed to identify and define at the molecular level the effects of pressure on the components of the signal transduction system in isolation and on the entire functional complex.

ACCOMPLISHMENTS: We have completed work characterizing the effects of pressure on the coupling efficiency of the A_1 adenosine receptor in brain membrane preparations (Siebenaller and Murray, 1990; Siebenaller et. al., 1991). For the teleost brain membrane preparations, incubation at 5° C and 476 atm does not result in loss of adenylyl cyclase activity or coupling to the A_1 adenosine receptor on subsequent assays at atmospheric pressure. In contrast, rat brain membrane preparations lost 59% of their activity under these conditions.

At atmospheric pressure, the K_m of 2-deoxy-ATP was identical for the Sebastolobus species adenylyl cyclase. Increased pressure increased the K_m values in both species. However, the K_m of 2-deoxy-ATP was less sensitive to pressure for the enzyme from the deeper-living S. altivelis. Basal adenylyl cyclase activity and the inhibitory effect of 100 μmol l^{-1} CPA were assayed at 1, 136 and 408 atm. Increased pressure inhibited basal adenylyl cyclase activity in both species. Basal adenylyl cyclase in brain membranes from the rat and 5 additional teleost species were also inhibited by increased pressure. At 136 atm CPA inhibited the adenylyl cyclase from both Sebastolobus species. However, at 408 atm the efficacy of CPA was reversed for S. altivelis, resulting in a stimulation of adenylyl cyclase activity. The phospholipid and fatty acid contents of brain membranes form the two Sebastolobus species do not differ. [^{32}P]ADP-ribosylation by pertussis toxin results in a 10- to 15-fold greater labeling of 39 and 41 kDa G-protein α subunits in brain membranes of S. altivelis. The G protein complement of these species may play a role in the differential pressure-sensitivity of signal transduction. To assess this possibility, studies are currently underway further characterizing the susceptibility of G proteins in the two species to [^{32}P]ADP-ribosylation, the content of immune reactive α and β subunits are being determined,

and the effects of hydrostatic pressure on the intrinsic GTPase activity of G protein α subunits are being characterized.

SIGNIFICANCE: The effects of hydrostatic pressure on membranes and membrane-associated systems may be the most extensive adaptational problems posed for organisms invading the deep sea. The general importance of pressure perturbation on membranes is seen in the high pressure neurological syndrome which has been demonstrated in a variety of taxa, including fishes. Our experiments indicate that pressure likely affects the enzyme effector and/or receptor-effector coupling in transmembrane signaling. Our studies also indicate that homeoviscous adaptation per se is not responsible for the differences in pressure response.

PUBLICATIONS AND ABSTRACTS

1. J.F. Siebenaller, A.F. Hagar and T.F. Murray: The effects of hydrostatic pressure on A_1 adenosine receptor signal transduction in brain membranes of two congeneric marine fishes. Journal of Experimental Biology, 159:23-43, 1991.

2. J.F. Siebenaller and T.F. Murray: A_1 adenosine receptor modulation of adenylyl cyclase of deep-living teleost fish, Antimora rostrata. Biological Bulletin 178:65-73, 1990.

3. Leid, M., Murray, T.F., Franklin, P.H. and Siebenaller, J.F.: A_1 adenosine receptor regulation of adenylyl cyclase in brain membranes of a deep-living teleost fish. Society for Neuroscience Abstracts, 15:233, 1989.

4. Murray, T.F., Blair, T.A., Leid, M., Franklin, P.H. and Siebenaller, J.F.: A_1 adenosine receptors in heart: Functional and biochemical consequences of activation. In: "Probing Bioactive Mechanisms." Edited by P. Magee, J. Block and D. Henry, ACS Symposium Series, American Chemical Society, Washington, DC, pp. 232-242, 1989.

5. Siebenaller, J.F. and Murray, T.F.: Evolutionary temperature adaptation of agonist binding to the A_1 adenosine receptor. Biological Bulletin, 175:410-416, 1988.